

Supplementary materials to

**Mitochondrial Genomic Dysfunction Causes Dephosphorylation of
Sch9 in the Yeast *Saccharomyces cerevisiae***

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Supplementary Table S1.

Supplementary Fig. S1.

Supplementary Table S1. Yeast strains used in this study.

Strains	Genotypes	Sources
TB50 α	MAT α <i>trp1 his3 ura3 leu2 rme1</i> [ρ^+]	Lab. stock
RL170-2b	TB50 α <i>TCO89-TAP[KlTrp1]</i> [ρ^+]	Lab. stock
YGSK226	TB50 α <i>TCO89-TAP[KlTrp1]</i> pJU676 pJU450 [ρ^+]	This study
YGSK232	TB50 α <i>TCO89-TAP[KlTrp1]</i> [ρ^0]	This study
YGSK242	TB50 α <i>TCO89-TAP[KlTrp1]</i> pJU676 pJU450 [ρ^0]	This study
YGSK269	TB50 α <i>TCO89-TAP[KlTrp1]</i> pJU675 pJU450 [ρ^+]	This study
YGSK270	TB50 α <i>TCO89-TAP[KlTrp1]</i> pJU841 pJU450 [ρ^+]	This study
YGSK271	TB50 α <i>TCO89-TAP[KlTrp1]</i> pJU675 pJU450 [ρ^0]	This study
YGSK272	TB50 α <i>TCO89-TAP[KlTrp1]</i> pJU841 pJU450 [ρ^0]	This study
TB50a	MATa <i>trp1 his3 ura3 leu2 rme1</i> [ρ^+]	Lab. stock
RL276-5D	TB50a <i>HIS3 LEU2</i> [ρ^+]	Lab. stock
YGSK239 ^a	TB50a <i>HIS3 LEU2</i> [ρ^0]	This study
YGSK197 ^a	TB50a <i>TCO89-TAP[KlTrp1] kog1Δ::kanMX6 snf1Δ::hph</i> YCplac111:: <i>KOG1</i> [ρ^+]	This study
BY4742	MAT α <i>leu2Δ0 lys2Δ0 ura3Δ0 his3Δ1</i> [ρ^+]	Euroscarf
YGSK229	BY4742 pJU676 pJU450 [ρ^+]	This study
YGSK233 ^a	BY4742 [ρ^0]	This study
YGSK243	BY4742 pJU676 pJU450 [ρ^0]	This study
BY4741	MATa <i>leu2Δ0 met15Δ0 ura3Δ0 his3Δ1</i> [ρ^+]	Euroscarf
YGSK238 ^a	BY4741 [ρ^0]	This study

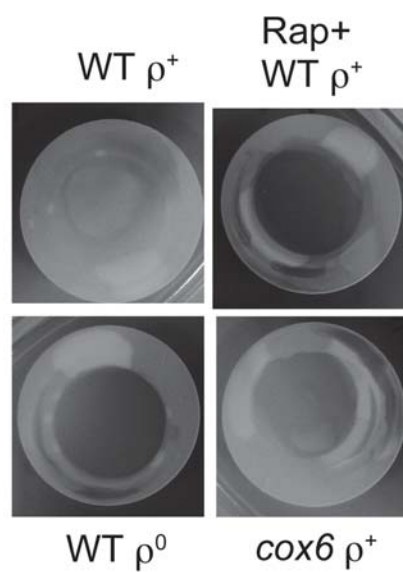
YGSK221	BY4741 <i>atp2Δ::kanMX4</i> [ρ^+]	Euroscarf
YGSK247	BY4741 <i>atp2Δ::kanMX4</i> pJU676 pJU450 [ρ^+]	This study
YGSK219	BY4741 <i>cox6Δ::kanMX4</i> [ρ^+]	Euroscarf
YGSK248 ^a	BY4741 <i>cox6Δ::kanMX4</i> pJU676 pJU450 [ρ^+]	This study
YGSK222	BY4741 <i>mrpl16Δ::kanMX4</i> [ρ^+]	Euroscarf
YGSK249	BY4741 <i>mrpl16Δ::kanMX4</i> pJU676 pJU450 [ρ^+]	This study
MK3306	BY4742 pRS315 pJU676 pRS413 [ρ^+]	This study
MK3307	BY4742 pRS315 pJU676 pRS413 [ρ^0]	This study
MK3309 ^b	BY4742 pPL132 (pRS315:: <i>TOR1</i>) pRS413 pJU450 [ρ^0]	This study
MK3311 ^b	BY4742 pPL156 (pRS315:: <i>TOR1</i> I1954V) pRS413 pJU450 [ρ^0]	This study
MK3313 ^b	BY4742 pPL158 (pRS315:: <i>TOR1</i> I1954V W2176R) pRS413 pJU450 [ρ^0]	This study
MK3354 ^c	BY4742 <i>gtr1Δ::kanMX4</i> YCplac33::pTetON-GTR1-Q65L pJU679 pRS413 [ρ^+]	This study
MK3355	BY4742 <i>gtr1Δ::kanMX4</i> YCplac33 pJU679 pRS413 [ρ^+]	This study
MK3356 ^c	BY4742 <i>gtr1Δ::kanMX4</i> YCplac33::pTetON-GTR1-Q65L pJU679 pRS413 [ρ^0]	This study
MK3357	BY4742 <i>gtr1Δ::kanMX4</i> YCplac33 pJU679 pRS413 [ρ^0]	This study

^a These strains were used for checking ρ^+ or ρ^0 phenotype.

^b Plasmids (pPL132, 156, and 158) were kindly gifted from Dr. Ted Powers (3).

^c Plasmid (YCplac33::pTetON-GTR1-Q65L) is described in ref (2).

A



B

phospho-Sch9 C-ter. {
Sch9 C-ter. -

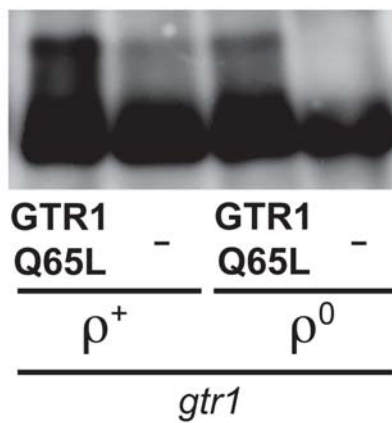


Fig. S1

Figure legend of Supplementary Fig. S1

FIG. S1 (A) Accumulation of glycogen. YGSK229 (WT ρ^+), YGSK243 (WT ρ^0), and YGSK248 (*cox6* ρ^+) (*cox6* ρ^+) cells were diluted to an A_{600} of 0.4 in fresh SD+Asn medium (containing: 2 % glucose, 10 mM Asn, 0.17 % yeast nitrogen base without ammonium sulfate [pH 5.6] plus 50 μ g/ml Lys for YGSK229 and YGSK243, 20 μ g/ml Met for YGSK248) and cultivated for approximately 4.5 h. YGSK229 cells were also cultivated in another flask as above for 3 h, and further cultivated for 1.5 h in the presence of 200 ng/ml rapamycin. 7.5 A_{600} equivalents of cells were filtered and treated with iodine vapour to stain glycogen as described (1). Dark staining indicates that glycogen was accumulated in; YGSK243 (WT ρ^0) and rapamycin-treated YGSK229 (Rap+ WT ρ^+) cells, but not in YGSK229 (WT ρ^+) and YGSK248 (*cox6* ρ^+) cells. (B) Sch9 is dephosphorylated independently of GTR1 in ρ^0 cells. MK3354 (*gtr1* YCplac33::pTetON-GTR1-Q65L ρ^+), MK3355 (*gtr1* YCplac33 ρ^+), MK3356 (*gtr1* YCplac33::pTetON-GTR1-Q65L ρ^0), and MK3357 (*gtr1* YCplac33 ρ^0) cells were cultured in SD+Asn medium containing 50 μ g/ml Lys and 5 μ g/ml doxycycline, diluted to an OD_{600} of 0.4 in the same medium, and further cultivated for 5.5 h. Sch9-phosphorylations were monitored as described in the text. Prior to SDS-PAGE / anti-HA blot, protein extracts were treated with NTCB to cleave proteins.

REFERENCES

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